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DATE: Thursday, February 16, 2006

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☐	L10	5858745.pn.	2
☐	L9	5659123.pn.	2
☐	L8	((mutat$ or mutant) with toxin) same ((cytotox$ or toxic or toxicity or kill or death) with increas$)	26
☐	L7	(select$ same (kill or death or cytotox$)) and l5	4
☐	L6	(select$ same (cell or cytotox$)) and l5	17
☐	L5	((mutat$ or mutant) with toxin) same ((cytotox$ or toxic or toxicity) with increas$)	18
☐	L4	((mutat$ or mutant) with toxin) same (cytotox$ with (increas$ or great$))	8
☐	L3	((mutat$ or mutant) with toxin) same (tox$ or cytotox$) same (increas$ or great$)	294
☐	L2	((mutat$ or mutant) with toxin) same (tox$ or cytotox$) same (increas$ or more or great$ or larg$)	762
	DB=PGPB,USPT; PLUR=YES; OP=OR		
☐	L1	((mutat$ or mutant) with toxin) same (tox$ or cytotox$) same (increas$ or more or great$ or larg$)	733

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=> ((mutat? or mutant) (s) toxin) and ((cytotox? or toxic or toxicity or kill or
death) (s) increas?)
L1          133 ((MUTAT? OR MUTANT) (S) TOXIN) AND ((CYTOTOX? OR TOXIC OR TOXICI
                TY OR KILL OR DEATH) (S) INCREAS?)

=> d scan l1

L1    133 ANSWERS   CAPLUS  COPYRIGHT 2006 ACS on STN
CC    4-5 (Toxicology)
TI    Dependence of ricin toxicity on translocation of the toxin A-chain from
      the endoplasmic reticulum to the cytosol
ST    ricin toxicity translocation endoplasmic reticulum cytosol
IT    Ricins
      RL: ADV (Adverse effect, including toxicity); BPR (Biological process);
      BSU (Biological study, unclassified); BIOL (Biological study); PROC
      (Process)
         (A; dependence of ricin toxicity on translocation of toxin A-chain from
         endoplasmic reticulum to cytosol)
IT    Cytoplasm
         (cytosol; dependence of ricin toxicity on translocation of toxin
         A-chain from endoplasmic reticulum to cytosol)
IT    Endoplasmic reticulum
      Golgi apparatus
         (dependence of ricin toxicity on translocation of toxin A-chain from
         endoplasmic reticulum to cytosol)
IT    Glycosylation
         (in endoplasmic reticulum; dependence of ricin toxicity on
         translocation of toxin A-chain from endoplasmic reticulum to cytosol)
IT    Biological transport
         (intracellular; dependence of ricin toxicity on translocation of toxin
         A-chain from endoplasmic reticulum to cytosol)
IT    Mutation
         (ricin A chain; dependence of ricin toxicity on translocation of
         toxin A-chain from endoplasmic reticulum to cytosol)
IT    7440-70-2, Calcium, biological studies
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); BIOL (Biological study)
         (dependence of ricin toxicity on translocation of toxin A-chain from
         endoplasmic reticulum to cytosol)

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):end

=> dup rem l1
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PROCESSING COMPLETED FOR L1
L2 68 DUP REM L1 (65 DUPLICATES REMOVED)

=> py>1998 and l2
L3 45 PY>1998 AND L2

=> l2 not l3
L4 23 L2 NOT L3

=> t ti l4 1-23

L4 ANSWER 1 OF 23 MEDLINE on STN
TI Stepwise transplantation of an active site loop between heat-labile enterotoxins LT-II and LT-I and characterization of the obtained hybrid toxins.

L4 ANSWER 2 OF 23 MEDLINE on STN
TI Evaluation of GM1 ganglioside-mediated apoptosis in feline thymocytes.

L4 ANSWER 3 OF 23 MEDLINE on STN
TI Increased sensitivity to mitochondrial **toxin**-induced apoptosis in neural cells expressing **mutant** presenilin-1 is linked to perturbed calcium homeostasis and enhanced oxyradical production.

L4 ANSWER 4 OF 23 MEDLINE on STN
TI Protein engineering of Bacillus thuringiensis delta-endotoxin: mutations at domain II of CryIAb enhance receptor affinity and toxicity toward gypsy moth larvae.

L4 ANSWER 5 OF 23 MEDLINE on STN
TI Pseudomonas exotoxin exhibits increased sensitivity to furin when sequences at the cleavage site are **mutated** to resemble the arginine-rich loop of diphtheria **toxin**.

L4 ANSWER 6 OF 23 MEDLINE on STN
TI An improved circularly permuted interleukin 4-toxin is highly cytotoxic to human renal cell carcinoma cells. Introduction of gamma c chain in RCC cells does not improve sensitivity.

L4 ANSWER 7 OF 23 MEDLINE on STN
TI Functional significance of loops in the receptor binding domain of Bacillus thuringiensis CryIIIA delta-endotoxin.

L4 ANSWER 8 OF 23 MEDLINE on STN
TI Cleavage of pseudomonas exotoxin and diphtheria toxin by a furin-like enzyme prepared from beef liver.

L4 ANSWER 9 OF 23 MEDLINE on STN
TI Alanine scanning mutagenesis identifies surface amino acids on domain II of Pseudomonas exotoxin required for cytotoxicity, proper folding, and secretion into periplasm.

L4 ANSWER 10 OF 23 MEDLINE on STN
TI Phe496 and Leu497 are essential for receptor binding and cytotoxic action of the murine interleukin-4 receptor targeted fusion toxin DAB389-mIL-4.

L4 ANSWER 11 OF 23 MEDLINE on STN
TI Characterization of **mutant** strains producing pertussis **toxin** cross reacting materials.

L4 ANSWER 12 OF 23 MEDLINE on STN
TI Enhancement of the cytotoxicity of mistletoe lectin-1 (ML-1) by high pH or

perturbation in Golgi functions.

L4 ANSWER 13 OF 23 MEDLINE on STN
TI Enhancement of immunotoxin efficacy by acid-cleavable cross-linking agents utilizing diphtheria toxin and toxin mutants.

L4 ANSWER 14 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI Use of the Vibrio cholerae irgA gene as a locus for insertion and expression of heterologous antigens in cholera vaccine strains.

L4 ANSWER 15 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI Heparin-binding transforming growth factor alpha Pseudomonas exotoxin A: A heparan sulfate-modulated recombinant toxin cytotoxic to cancer cells and proliferating smooth muscle cells.

L4 ANSWER 16 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI PROPERTIES OF CHIMERIC TOXINS WITH TWO RECOGNITION DOMAINS INTERLEUKIN 6 AND TRANSFORMING GROWTH FACTOR ALPHA AT DIFFERENT LOCATIONS IN PSEUDOMONAS EXOTOXIN.

L4 ANSWER 17 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI MUTAGENICITY OF QUERCETIN IN CHINESE HAMSTER LUNG CELLS IN CULTURE.

L4 ANSWER 18 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI GENETIC EFFECTS OF PR TOXIN ON PROKARYOTIC AND EUKARYOTIC MICROORGANISMS.

L4 ANSWER 19 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI CYTO TOXICITY AND ABSENCE OF MUTAGENIC ACTIVITY OF VOMI **TOXIN** 4 DEOXY NIVALENOL IN A HEPATOCYTE MEDIATED **MUTATION** ASSAY WITH V-79 CHINESE HAMSTER LUNG CELLS.

L4 ANSWER 20 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI TESTS FOR MUTAGENICITY IN SALMONELLA AND COVALENT BINDING TO DNA AND PROTEIN IN THE RAT OF THE RIOT CONTROL AGENT O CHLOROBENZYLIDENE MALONONITRILE.

L4 ANSWER 21 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
TI Mutant cytokines having increased receptor affinity

L4 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
TI Construction by site-directed mutagenesis of a 39-kilodalton mosquitocidal protein similar to the larva-processed toxin of Bacillus sphaericus 2362

L4 ANSWER 23 OF 23 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI Modified Bacillus thuringiensis CryIII proteins - with **increased toxicity** against insect pests, particularly Coleopteran insects, e.g. corn rootworm and Colorado potato beetle.

=> d ibib abs l4 1-23

L4 ANSWER 1 OF 23 MEDLINE on STN
ACCESSION NUMBER: 1999092760 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9876933
TITLE: Stepwise transplantation of an active site loop between

heat-labile enterotoxins LT-II and LT-I and characterization of the obtained hybrid toxins.

AUTHOR: Feil I K; Platas A A; van den Akker F; Reddy R; Merritt E A; Storm D R; Hol W G

CORPORATE SOURCE: Howard Hughes Medical Institute, Department of Biological Structure, University of Washington, Seattle 98195-7742, USA.

CONTRACT NUMBER: AI 34501 (NIAID)

SOURCE: Protein engineering, (1998 Nov) 11 (11) 1103-9.
Journal code: 8801484. ISSN: 0269-2139.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199903

ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990308

AB Members of the cholera toxin family, including Escherichia coli heat-labile enterotoxins LT-I and LT-II, catalyze the covalent modification of intracellular proteins by transfer of ADP-ribose from NAD to a specific arginine of the target protein. The ADP-ribosylating activity of these toxins is located in the A-subunit, for which LT-I and LT-II share a 63% sequence identity. The flexible loop in LT-I, ranging from residue 47 to 56, closes over the active site cleft. Previous studies have shown that point mutations in this loop have dramatic effects on the activity of LT-I. Yet, in LT-II the sequence of the equivalent loop differs at four positions from LT-I. Therefore five mutants of the active site loop were created by a stepwise replacement of the loop sequence in LT-I with virtually all the corresponding residues in LT-II. Since we discovered that LT-II had no activity versus the artificial substrate diethylamino-benzylidine-aminoguanidine (DEABAG) while LT-I does, our active site mutants most likely probe the NAD binding, not the arginine binding region of the active site. The five hybrid toxins obtained (Q49A, F52N, V53T, Q49V/F52N and Q49V/F52N/V53T) show (i) great differences in holotoxin assembly efficiency; (ii) decreased **cytotoxicity** in Chinese hamster ovary cells; and (iii) **increased** in vitro enzymatic activity compared with wild type LT-I. Specifically, the three mutants containing the F52N substitution display a greater Vmax for NAD than wild type LT-I. The enzymatic activity of the V53T mutant is significantly higher than that of wild type LT-I. Apparently this subtle variation at position 53 is beneficial, in contrast to several other substitutions at position 53 which previously had been shown to be deleterious for activity. The most striking result of this study is that the active site loop of LT-I, despite great sensitivity for point **mutations**, can essentially be replaced by the active site loop of LT-II, yielding an active 'hybrid enzyme' as well as 'hybrid **toxin**'.

L4 ANSWER 2 OF 23 MEDLINE on STN

ACCESSION NUMBER: 1999061475 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9847018

TITLE: Evaluation of GM1 ganglioside-mediated apoptosis in feline thymocytes.

AUTHOR: Zhou J; Cox N R; Ewald S J; Morrison N E; Basker H J

CORPORATE SOURCE: Department of Internal Medicine, St. Luke's Hospital, St. Louis, MO 63017, USA.

CONTRACT NUMBER: 1R15AI34129-01A1 (NIAID)

SOURCE: Veterinary immunology and immunopathology, (1998 Nov 6) 66 (1) 25-42.
Journal code: 8002006. ISSN: 0165-2427.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990301
Last Updated on STN: 20000303
Entered Medline: 19990217

AB Cats with inherited GM1 gangliosidosis (GM1 mutant cats) have premature thymic involution characterized by decreased total thymocytes primarily affecting the CD4+ CD8+ subpopulation. While GM1 **mutant** cats have increased cell surface GM1 gangliosides, as determined by cholera **toxin** B binding, on both thymocytes and peripheral lymph node cells only thymocytes show increased apoptosis. To determine if GM1 gangliosides can increase the occurrence of apoptosis in feline thymocytes directly, we added exogenous GM1 ganglioside (GM1) to feline thymocyte primary cultures and compared the results to apoptotic changes seen in untreated cells or in cells treated with dexamethasone (Dex), a known inducer of thymocyte apoptosis in other species. Incorporation of exogenous GM1 into thymocyte cytoplasmic membranes was confirmed by flow cytometric analyses of cholera toxin B labelling. Apoptosis in feline thymocytes was analyzed by electron microscopy, spectrophotometric evaluation of DNA fragmentation, flow cytometric enumeration of apoptotic nuclei, and gel electrophoretic analysis of degraded DNA. Alterations in percentages of thymocyte immunophenotype following GM1 incorporation were determined by flow cytometric analyses of labelled cell surface markers for feline CD4 and CD8. Because in vitro addition of GM1 gangliosides has been reported in other species to decrease surface expression of CD4 on both thymocytes and peripheral lymphocytes, we evaluated GM1-associated down-regulation of CD4 on the surface of feline thymocytes and peripheral lymph node cells by flow cytometry. Additionally, we compared the apoptotic response of the more mature peripheral lymph node cells to the less mature thymocytes. Our results indicate that incorporation of exogenous GM1 into feline thymocyte cell membranes produces a dose-dependent **increase** of apoptotic cell **death**.
Although, CD4 expression on both feline thymocyte and lymph node cell membranes was abruptly decreased after introducing exogenous GM1, enhanced apoptotic death was observed only in thymocytes, not in lymph node cells at the same GM1 concentration. Enhancement of thymocyte apoptosis appears to be age-related since cells derived from cats <3 months of age were more vulnerable than those from cats >3 months of age.

L4 ANSWER 3 OF 23 MEDLINE on STN
ACCESSION NUMBER: 1998279057 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9614221
TITLE: Increased sensitivity to mitochondrial **toxin** -induced apoptosis in neural cells expressing **mutant** presenilin-1 is linked to perturbed calcium homeostasis and enhanced oxyradical production.
AUTHOR: Keller J N; Guo Q; Holtsberg F W; Bruce-Keller A J; Mattson M P
CORPORATE SOURCE: Molecular and Cellular Biology Group, Department of Biology, University of Kentucky, Lexington, Kentucky 40536, USA.
CONTRACT NUMBER: AG05119 (NIA)
AG14554 (NIA)
NS35253 (NINDS)
+
SOURCE: Journal of neuroscience : official journal of the Society for Neuroscience, (1998 Jun 15) 18 (12) 4439-50.
Journal code: 8102140. ISSN: 0270-6474.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980716
Last Updated on STN: 20000303
Entered Medline: 19980708

AB Many cases of autosomal dominant early onset Alzheimer's disease (AD) result from mutations in the gene encoding presenilin-1 (PS-1). PS-1 is an integral membrane protein expressed ubiquitously in neurons throughout the brain in which it is located primarily in endoplasmic reticulum (ER). Although the pathogenic mechanism of PS-1 mutations is unknown, recent findings suggest that PS mutations render neurons vulnerable to apoptosis. Because **increasing** evidence indicates that mitochondrial alterations contribute to neuronal **death** in AD, we tested the hypothesis that PS-1 mutations sensitize neurons to mitochondrial failure. PC12 cell lines expressing a PS-1 mutation (L286V) exhibited increased sensitivity to apoptosis induced by 3-nitropropionic acid (3-NP) and malonate, inhibitors of succinate dehydrogenase, compared with control cell lines and lines overexpressing wild-type PS-1. The apoptosis-enhancing action of mutant PS-1 was prevented by antioxidants (propyl gallate and glutathione), zVAD-fmk, and cyclosporin A, indicating requirements of reactive oxygen species (ROS), caspases, and mitochondrial permeability transition in the cell death process. 3-NP induced a rapid elevation of [Ca2+]i, which was followed by caspase activation, accumulation of ROS, and decreases in mitochondrial reducing potential and transmembrane potential in cells expressing mutant PS-1. The calcium chelator BAPTA AM and agents that block calcium release from ER and influx through voltage-dependent channels prevented mitochondrial ROS accumulation and membrane depolarization and apoptosis. Our data suggest that by perturbing subcellular calcium homeostasis presenilin mutations sensitize neurons to mitochondria-based forms of apoptosis that involve oxidative stress.

L4 ANSWER 4 OF 23 MEDLINE on STN
ACCESSION NUMBER: 97121386 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8962052
TITLE: Protein engineering of Bacillus thuringiensis delta-endotoxin: mutations at domain II of CryIAb enhance receptor affinity and toxicity toward gypsy moth larvae.
AUTHOR: Rajamohan F; Alzate O; Cotrill J A; Curtiss A; Dean D H
CORPORATE SOURCE: Department of Biochemistry, Ohio State University, Columbus 43210-1292, USA.
CONTRACT NUMBER: RO1 AI 29092 (NIAID)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1996 Dec 10) 93 (25) 14338-43. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970115

AB Substitutions or deletions of domain II loop residues of Bacillus thuringiensis delta-endotoxin CryIAb were constructed using site-directed mutagenesis techniques to investigate their functional roles in receptor binding and toxicity toward gypsy moth (Lymantria dispar). Substitution of loop 2 residue N372 with Ala or Gly (N372A, N372G) **increased** the **toxicity** against gypsy moth larvae 8-fold and enhanced binding affinity to gypsy moth midgut brush border membrane vesicles (BBMV) approximately 4-fold. Deletion of N372 (D3), however,

substantially reduced toxicity (> 21 times) as well as binding affinity, suggesting that residue N372 is involved in receptor binding. Interestingly, a triple **mutant**, DF-1 (N372A, A282G and L283S), has a 36-fold **increase** in **toxicity** to gypsy moth neonates compared with wild-type **toxin**. The enhanced activity of DF-1 was correlated with higher binding affinity (18-fold) and binding site concentrations. Dissociation binding assays suggested that the off-rate of the BBMV-bound mutant toxins was similar to that of the wild type. However, DF-1 toxin bound 4 times more than the wild-type and N372A toxins, and it was directly correlated with binding affinity and potency. Protein blots of gypsy moth BBMV probed with labeled N372A, DF-1, and CryIAb toxins recognized a common 210-kDa protein, indicating that the increased activity of the mutants was not caused by binding to additional receptor(s). The improved binding affinity of N372A and DF-1 suggest that a shorter side chain at these loops may fit the toxin more efficiently to the binding pockets. These results offer an excellent model system for engineering delta-endotoxins with higher potency and wider spectra of target pests by improving receptor binding interactions.

L4 ANSWER 5 OF 23 MEDLINE on STN
ACCESSION NUMBER: 97109543 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8951823
TITLE: Pseudomonas exotoxin exhibits increased sensitivity to furin when sequences at the cleavage site are **mutated** to resemble the arginine-rich loop of diphtheria **toxin**.
AUTHOR: Chiron M F; Ogata M; FitzGerald D J
CORPORATE SOURCE: Biotherapy Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: Molecular microbiology, (1996 Nov) 22 (4) 769-78. Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970414
Last Updated on STN: 19970414
Entered Medline: 19970331

AB To be toxic for mammalian cells, Pseudomonas exotoxin (PE) requires proteolytic cleavage between Arg-279 and Gly-280. Cleavage, which is mediated by the cellular protease furin, generates an active C-terminal fragment which translocates to the cytosol and inhibits protein synthesis. In vitro, furin-mediated cleavage is optimal at pH 5.5 with a relatively slow turnover rate. Within cells, only 5-10% of cell-associated PE is cleaved. To investigate the reasons for this inefficient cleavage, the amino acid composition near the cleavage site was altered to resemble more closely the arginine-rich sequence from the functionally similar region of diphtheria toxin (DT). Four PE-DT mutants were generated, whereby 1, 5, 6 or 8 amino acids at the PE-cleavage site were changed to amino acids found at the DT-cleavage site. Mutant proteins were expressed in Escherichia coli, purified and then analysed for their susceptibility to cleavage by furin and trypsin, susceptibility to cell-mediated cleavage, and cytotoxic activity relative to wild-type PE. At pH 5.5, the rate of both furin-mediated cleavage and trypsin-mediated cleavage increased dramatically when amino acids in PE were altered to resemble the DT sequence. This increase did not alter the pH optimum for furin-mediated cleavage of PE toxins, which remained at pH 5.0-5.5. When radioactive versions of selected PE-DT proteins were added to intact cells, an increase in the percentage of molecules that were cleaved relative to wild-type PE was also seen. However, changes that favoured **increased** proteolysis apparently interfered with other important

toxin functions because none of the PE-DT proteins exhibited enhanced **toxicity** for cells when compared with the activity of wild-type PE.

L4 ANSWER 6 OF 23 MEDLINE on STN
ACCESSION NUMBER: 96289937 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8660841
TITLE: An improved circularly permuted interleukin 4-toxin is highly cytotoxic to human renal cell carcinoma cells. Introduction of gamma c chain in RCC cells does not improve sensitivity.
AUTHOR: Puri R K; Leland P; Obiri N I; Husain S R; Mule J; Pastan I; Kreitman R J
CORPORATE SOURCE: Laboratory of Molecular Tumor Biology, Food and Drug Administration, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: Cellular immunology, (1996 Jul 10) 171 (1) 80-6. Journal code: 1246405. ISSN: 0008-8749.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20020420
Entered Medline: 19961122

AB We have previously demonstrated that a chimeric protein composed of human IL-4 and Pseudomonas exotoxin, termed IL4-PE4E, is cytotoxic to primary cells derived from human renal cell carcinoma (RCC). To improve the cytotoxicity of IL4-toxins such as IL4-PE4E and IL4-PE38KDEL to IL-4 receptor (IL-4R) positive tumor cells, a circularly permuted chimeric **toxin** was prepared by fusing a truncated PE gene encoding PE38KDEL 3' to a circularly permuted IL-4 **mutant** gene encoding IL4 amino acids 38-129, the linker GGNGG, and IL4 amino acids 1-37. The resulting chimeric protein, termed IL4(38-37)-PE38KDEL, was tested on five RCC cell lines and its cytotoxicity was compared to that of the native IL4-toxins IL4-PE4E and IL4-PE38KDEL. IL4(38-37)-PE38KDEL was found to be 5 to 10 times more cytotoxic to all cell cultures tested compared to either native IL4-toxin. The cytotoxic activity of IL4(38-37)-PE38KDEL was competible by excess IL-4 and was confirmed by clonogenic assay. IL4(38-37)-PE38KDEL bound to IL-4R on RCC cells with 6- to 12-fold higher affinity than IL4-PE38KDEL or IL4-PE4E. RCC tumor cells were found to lack the common gamma chain (gamma c) of the IL-4R reported to be present on immune cells. The stable transfection of RCC cells with the gamma c chain gene did not significantly change their sensitivity to IL4(38-37)-PE38KDEL. Taken together, our results indicate that the CPIL4-toxin IL4(38-37)-PE38KDEL is highly **cytotoxic** to human RCC cells due to **increased** binding affinity to IL-4R while it is not **cytotoxic** or slightly **cytotoxic** to T and B cells, monocytic cell lines, and fresh resting or activated bone marrow-derived cells. The gamma c does not seem to increase the internalization rate and/or processing of IL4-toxins in RCC cells. CPIL4-toxin may be a useful agent for the treatment of human RCC.

L4 ANSWER 7 OF 23 MEDLINE on STN
ACCESSION NUMBER: 96163559 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8568902
TITLE: Functional significance of loops in the receptor binding domain of Bacillus thuringiensis CryIIIA delta-endotoxin.
AUTHOR: Wu S J; Dean D H
CORPORATE SOURCE: Department of Biochemistry, Ohio State University, Columbus 43210, USA.

CONTRACT NUMBER: RO1 A1 29092
SOURCE: Journal of molecular biology, (1996 Feb 2) 255 (4) 628-40.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960315
Last Updated on STN: 19960315
Entered Medline: 19960307

AB Analysis of the three surface loops in domain II of Bacillus thuringiensis CryIIIA delta-endotoxin has been carried out to assess their role in receptor binding and toxicity. Site-directed mutagenesis was used to convert loop residues to alanine and the mutant proteins were analyzed for structural stability, toxicity to beetle larvae (Tenebrio molitor), binding to receptors on T. molitor brush border membrane vesicles (Tm-BBMV) and insertion into BBMV, as measured by irreversible membrane receptor binding. This study demonstrates the functional significance of loops for binding and insertion. Alanine replacements in loop I resulted in disruption of receptor binding or structural instability. The double mutation, Y350A, Y351A, could be suppressed by replacing a nearby R345 with alanine, and the resultant mutant protein also showed reduced receptor binding. Substitution of N353 and D354 in loop I with alanine residues caused the loss of binding ability and toxicity. A loop II double mutant, P412A, S413A, had no effect on binding or toxicity. A block mutation of loop III residues to alanine had the effect of reducing receptor binding while concomitantly **increasing toxicity** by 2.4-fold. We compared this up-**mutant** to wild-type **toxin** in each step of physiological processing of protoxin: solubility, proteolytic activation, and insertion into the Tm-BBMV. The loop III block **mutant** showed increased membrane insertion, but was similar to wild-type **toxin** in other parameters. These results reveal that loop I and loop III in domain II of CryIIIA delta-endotoxin are involved in receptor binding. In addition, the direct correlation between toxicity and irreversible binding of the loop III block mutant (despite the indirect relationship to reversible binding) suggests that loop III may play a role in membrane insertion.

L4 ANSWER 8 OF 23 MEDLINE on STN
ACCESSION NUMBER: 94299536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8027078
TITLE: Cleavage of pseudomonas exotoxin and diphtheria toxin by a furin-like enzyme prepared from beef liver.
AUTHOR: Chiron M F; Fryling C M; FitzGerald D J
CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892.
CONTRACT NUMBER: CA-12197 (NCI)
RR-04869 (NCRR)
SOURCE: Journal of biological chemistry, (1994 Jul 8) 269 (27) 18167-76.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940818
Last Updated on STN: 20020420
Entered Medline: 19940808

AB Pseudomonas exotoxin (PE) is cleaved within mammalian cells between Arg279 and Gly280 to generate an enzymatically active COOH-terminal fragment of

37 kDa which translocates to the cytosol and ADP-ribosylates elongation factor 2. A protease, with toxin cleaving activity, was prepared from beef liver and subsequently characterized. After achieving a 500-fold enrichment in several chromatographic steps, a soluble form of this protease was identified as a furin-like enzyme. It cleaved PE on the COOH-terminal side of the sequence of RQPR (amino acids 276-279) producing the same fragments as those generated within cells. Cleavage had a pH optimum of 5.0-5.5, was inhibited by EDTA or p-hydroxymercuribenzoate but not by O-phenanthroline,N-ethylmaleimide, trans-epoxysuccinyl-L-leukcylamido-(4-guanidino)-butane, or PMSF (or other well known inhibitors of serine proteases). The beef protease cleaved PE with an apparent Km of 7 microM. A **mutant** form of PE, PEala281, was cleaved at the same site, with the same pH optimum, a similar Km (9 microM) but with a Vmax 150 times faster than was seen with the native **toxin**. Mutational analysis of the amino acids located just before the site of cleavage, confirmed the importance of arginines at P-1 and P-4. It was also noted that the introduction of a dibasic pair at 278-279 did not **increase toxicity** or appreciably improve the rate of cleavage. Unnicked diphtheria toxin (DT) was also cleaved by the beef protease; cleavage was on the COOH-terminal side of the sequence RVRR (amino acids 190-193), was seen at pH values ranging from 5.5 to 8.5 and had an optimum at pH 8.0. Recombinant furin cleaved PE, PEala281, and DT with the same characteristics as the beef protease. In addition, Western blot analysis revealed that anti-furin antibodies reacted specifically with components in the beef protease preparation. Immunodepletion experiments showed that all toxin-cleavage activity could be removed from the beef protease using anti-furin antibodies. The relevance of furin-mediated cleavage was further assessed by adding nicked toxins to intact cells. Nicked PE and DT both killed cells at a faster rate than their unnicked counterparts.

L4 ANSWER 9 OF 23 MEDLINE on STN
ACCESSION NUMBER: 93054682 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1429683
TITLE: Alanine scanning mutagenesis identifies surface amino acids on domain II of Pseudomonas exotoxin required for cytotoxicity, proper folding, and secretion into periplasm.
AUTHOR: Kasturi S; Kihara A; FitzGerald D; Pastan I
CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: Journal of biological chemistry, (1992 Nov 15) 267 (32) 23427-33.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 20020420
Entered Medline: 19921216

AB Pseudomonas exotoxin A (PE) is a single polypeptide chain that contains 613 amino acids and is arranged into three major structural domains. Domain Ia is responsible for cell recognition, domain II for translocation of PE across the membrane, and domain III for ADP-ribosylation of elongation factor 2. Recombinant PE can be produced in Escherichia coli and is efficiently secreted into the periplasm when an OmpA signal sequence is present. To investigate the role of the amino acids located on the surface of domain II in the action of the toxin against mammalian cells, we substituted alanine for each of the 27 surface amino acids present in domain II. Surprisingly, all 27 mutant proteins had some alteration in cytotoxicity when tested on human A431 or MCF7 cells or

mouse L929 cells. Native PE has a compact structure and therefore is relatively protease resistant and very little ADP-ribosylation activity is detected in the absence of the denaturing agents like urea and dithiothreitol. Several of the **mutations** resulted in altered protease sensitivity of the **toxin**. Seven of the mutant molecules exhibited ADP-ribosylation activity without urea and dithiothreitol, indicating they are partially unfolded. Out of these seven mutants, six had **increased cytotoxic** activity on at least one of the target cell lines and the other retained its native **cytotoxic** potency.

L4 ANSWER 10 OF 23 MEDLINE on STN
ACCESSION NUMBER: 93028322 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1409544
TITLE: Phe496 and Leu497 are essential for receptor binding and cytotoxic action of the murine interleukin-4 receptor targeted fusion toxin DAB389-mIL-4.
AUTHOR: Lakkis F; Landgraf B; Wen Z; Strom T B; Murphy J R
CORPORATE SOURCE: Evans Department of Clinical Research, University Hospital, Boston, MA 02118.
CONTRACT NUMBER: U01 CA-48626 (NCI)
SOURCE: Protein engineering, (1992 Apr) 5 (3) 241-8.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19980206
Entered Medline: 19921103

AB DAB389-mIL-4 is a murine interleukin-4 (mIL-4) diphtheria toxin-related fusion protein which has been shown to be selectively toxic to cells expressing the mIL-4 receptor. In this report, we have used site-directed and in-frame deletion mutagenesis to study the role of the putative C-terminal alpha-helix (helix E) of the mIL-4 component of DAB389-mIL-4 in the intoxication process. We demonstrate that deletion of the C-terminal 15 amino acids of the fusion toxin leads to loss of cytotoxicity. The substitution of Phe496 with either Pro, Ala or Tyr, results in a greater than 20-fold decrease in cytotoxic activity of the respective mutant fusion toxins. In addition, substitution of Leu497 with either Ala or Glu results in a similar loss of cytotoxic activity. All of these **mutant** forms of the mIL-4 fusion **toxin** demonstrate a significant decrease in binding affinity (Ki) to the mIL-4 receptor in a competitive radioligand binding assay. In marked contrast, however, the substitution of Asp495 with Asn results in a 4-fold **increase** in **cytotoxic** potency and binding affinity to mIL-4 receptor bearing cells in vitro.

L4 ANSWER 11 OF 23 MEDLINE on STN
ACCESSION NUMBER: 92137529 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1778339
TITLE: Characterization of **mutant** strains producing pertussis **toxin** cross reacting materials.
AUTHOR: Sato Y; Sato H; Chazono M; Ginnaga A; Tamura C
CORPORATE SOURCE: National Institute of Health, Tokyo, Japan.
SOURCE: Developments in biological standardization, (1991) 73 93-107.
Journal code: 0427140. ISSN: 0301-5149.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920329
Last Updated on STN: 20021218
Entered Medline: 19920310

AB We have isolated 120 **mutant** strains producing pertussis **toxin** (PT) cross reacting materials (CRMs) from B. pertussis, strain Tohama, phase I by nitrosoguanidine treatment. Strains producing higher PT tend to show higher virulence in mice. No direct correlation between the virulence and other factors, such as filamentous hemagglutinin, adenylate cyclase or dermonecrotic heat labile toxin, was found. Most CRMs were less reactive to the anti-S1 monoclonal antibody, 1B7. When the PT CRMs produced by strains 69D, 74E or 79G, which were less or non-**toxic**, were mixed with A protomer purified from native PT, the PT activity assayed by clustering of CHO-cells **increased** significantly, but not when they were mixed with B oligomer. These CRMs may be composed of defective S1 and intact S2, S3, S4 and S5. Molecular sizes of PT CRMs outside and inside the cells were analysed by sucrose density gradient centrifugation. The sizes of the CRMs were in the range of 10K to 210K, but the biological activity of PT was detected at only the same molecular size, 106 K, as native PT. The majority of the CRM was released into culture medium if all five subunits were assembled; otherwise they accumulated inside the cell without completion of assembly to form the hexamer in the PT-form. One of the non-toxic mutants named 79G showed one point mutation from G to A at the 730th base from the Eco R1 site of the PT gene. Replacement of Cys-41 with Tyr-41 in S1 must have resulted from this mutation. 79G PT composed of S234 (5) was accumulated both inside and outside the cells because the mutant S1 could not form the disulfide bond in the molecule to form the hexamer with the B oligomer, and also S1 must be degraded because of its instability in the cells. Nevertheless 79 GPT showed high immunoprotectivity in mice by active or passive immunization against ic or aerosol challenge with B. pertussis, strain 18323, respectively. It may have a proper conformational structure for protective immunogenicity and could become a good candidate strain for production of a safer and effective pertussis vaccine in the future.

L4 ANSWER 12 OF 23 MEDLINE on STN
ACCESSION NUMBER: 91376132 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1910176
TITLE: Enhancement of the cytotoxicity of mistletoe lectin-1 (ML-1) by high pH or perturbation in Golgi functions.
AUTHOR: Yoshida T; Zhang M; Chen C; Franz H; Wu H C
CORPORATE SOURCE: Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland.
CONTRACT NUMBER: GM28810 (NIGMS)
SOURCE: Die Pharmazie, (1991 May) 46 (5) 349-51.
Journal code: 9800766. ISSN: 0031-7144.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199110
ENTRY DATE: Entered STN: 19911108
Last Updated on STN: 20021217
Entered Medline: 19911018

AB We have studied the cytotoxicity of Mistletoe lectin-1 (ML-1), a cytotoxic protein produced by Viscum album, in CHO and V79 cells and in mutant cell lines altered in Golgi functions or in endosomal acidification. In wild-type CHO cells, cytotoxicity of ML-1 was greatly enhanced by ammonium chloride or nigericin. A CHO **mutant** defective in endosomal acidification (DMPR-2), which is resistant to diphtheria **toxin**,

modeccin and Pseudomonas aeruginosa exotoxin A and hypersensitive to ricin, showed increased sensitivity to ML-1. MonR-31 and MF-1 are monensin- and compactin-resistant mutants derived from CHO and V79 cell lines, respectively, and are presumably altered in Golgi functions. The **cytotoxicity** of ML-1 was found to be **increased** in both MonR-31 and MF-1 cells as compared with their parental cells. These results indicate that the effects of chemicals or mutations altering endosomal acidification and Golgi functions on the cytotoxicity of ML-1 are similar to those on ricin cytotoxicity. Our results suggest that the **cytotoxicity** of ML-1 is enhanced by an **increase** in endosomal pH, as well as by chemicals or mutations altering the structure/functions of the Golgi regions. Like ricin, the intoxication process of ML-1 may involve the Golgi regions.

L4 ANSWER 13 OF 23 MEDLINE on STN
ACCESSION NUMBER: 89359255 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2475487
TITLE: Enhancement of immunotoxin efficacy by acid-cleavable cross-linking agents utilizing diphtheria toxin and toxin mutants.
AUTHOR: Neville D M Jr; Srinivasachar K; Stone R; Scharff J
CORPORATE SOURCE: Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, Maryland 20892.
SOURCE: Journal of biological chemistry, (1989 Sep 5) 264 (25) 14653-61.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198910
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19960129
Entered Medline: 19891011

AB We have utilized a new class of acid-cleavable protein cross-linking reagents in the construction of antibody-diphtheria toxin conjugates (Srinivaschar, K., and Neville, D. M., Jr. (1989) Biochemistry 28, 2501-2509). The potency of anti-CD5 conjugates assayed by inhibition of protein synthesis on CD5 bearing cells (Jurkat) is correlated with cross-linker hydrolytic rates. The maximum increase in potency of the cleavable conjugates over non-cleavable conventional conjugates is 50-fold and is specific for the CD5 uptake route as judged by competition with excess anti-CD5. The potency of conjugates made from diphtheria toxin and the anti-high molecular weight melanoma-associated antigen (HMW-MAA) is enhanced 3-10-fold by a cleavable cross-linker. However the potency of transferrin or anti-CD3 diphtheria toxin conjugates is only minimally enhanced (2-3-fold). **Mutant** diphtheria toxins, CRM103 and CRM9, previously shown to express less than 1/100 of the wild type in binding affinity were substituted into these conjugates as probes for possible intracellular **toxin** receptor interactions. Both mutants were equally as toxic to Jurkat target cells exhibiting 1/700 the wild-type potency. CRM9 non-cleavable conjugates were equally as potent as wild-type conjugates for transferrin and anti-CD3-mediated uptake but not for anti-CD5-mediated uptake where toxicity was reduced 60-fold over the wild-type analog. The cleavable cross-linker enhanced the toxicity of anti-CD5-CRM103 and anti-CD5-CRM9 conjugates, but potency was only 1/10 that of the analogous wild-type cleavable conjugate. These data are consistent with a model in which potentiation of **toxicity** of the anti-CD5 and anti-high molecular weight melanoma-associated antigen conjugates by the cleavable cross-linker occurs from an enhanced intracellular toxin-toxin receptor interaction that ultimately results in **increased** toxin translocation to the cytosol compartment. In

contrast, these data indicate that the anti-CD3 and transferrin uptake systems do not require this interaction in agreement with previous work (Johnson, V.G., Wilson, D., Greenfield, L., and Youle, R. J. (1988) J. Biol. Chemical 263, 1295-1300).

L4 ANSWER 14 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1994:18123 BIOSIS
DOCUMENT NUMBER: PREV199497031123
TITLE: Use of the Vibrio cholerae irgA gene as a locus for insertion and expression of heterologous antigens in cholera vaccine strains.
AUTHOR(S): Butterton, Joan R.; Boyko, Stephanie A.; Calderwood, Stephen B. [Reprint author]
CORPORATE SOURCE: Dep. Microbiol. Mol. Genet., Harvard Med. Sch., Boston, MA 02115, USA
SOURCE: Vaccine, (1993) Vol. 11, No. 13, pp. 1327-1335. CODEN: VACCDE. ISSN: 0264-410X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Jan 1994
Last Updated on STN: 25 Jan 1994

AB Vibrio cholerae may be a particularly effective organism for use in delivering heterologous antigens to stimulate a common mucosal immune response. A live attenuated vaccine strain of V. cholerae was constructed from the ctxA deletion **mutant** 0395-N1, containing the B subunit of Shiga-like **toxin** I under the transcriptional control of the iron-regulated irgA promoter. The B subunit of Shiga-like toxin I is identical to the B subunit of Shiga toxin (StxB). irgA encodes the major iron-regulated outer membrane protein of V. cholerae, which is a known virulence factor for this organism. Clones of the structural gene irgA from the classical V. cholerae strain 0395, with the gene for the Shiga-like toxin I B subunit inserted under the control of the irgA promoter, were used to introduce an internal deletion of irgA into the chromosome of 0395-N1 by in vivo marker exchange, using the suicide vector plasmid pCVD442. This plasmid contains the sacB gene from Bacillus subtilis, which allowed positive selection for loss of plasmid sequences on exposure to sucrose. The construction of vaccine strains was confirmed by Southern hybridization studies and outer membrane protein analysis. The expression of StxB in the vaccine strain VAC2 following growth in high- or low-iron conditions was shown to be tightly iron-regulated by Western blot analysis and by quantification of StxB using a sandwich enzyme-linked immunosorbent assay. The production of StxB by VAC2 under low-iron conditions was greater than that of the reference strain Shigella dysenteriae 60R. This vaccine strain produced no detectable **cytotoxicity** in a HeLa cell assay, and showed no **increased** virulence over the attenuated parent strain, 0395-N1, in a suckling mouse model. We suggest that the V. cholerae irgA gene is a particularly useful locus for the insertion and expression of heterologous antigens in cholera vaccine strains for oral delivery.

L4 ANSWER 15 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1993:209825 BIOSIS
DOCUMENT NUMBER: PREV199395111050
TITLE: Heparin-binding transforming growth factor alpha Pseudomonas exotoxin A: A heparan sulfate-modulated recombinant toxin cytotoxic to cancer cells and proliferating smooth muscle cells.
AUTHOR(S): Mesri, Enrique A.; Kreitman, Robert J.; Fu, Ya-Min; Epstein, Stephen E.; Pastan, Ira [Reprint author]
CORPORATE SOURCE: Lab. Molecular Biol., National Cancer Inst., National Inst.

Health, 9000 Rockville Pike, Build. 37, Room 4E16, Bethesda, Md 20892, USA

SOURCE: Journal of Biological Chemistry, (1993) Vol. 268, No. 7, pp. 4853-4862.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Apr 1993
Last Updated on STN: 9 Jun 1993

AB TGF-alpha-PE40, a recombinant **toxin** in which transforming growth factor alpha (TGF-alpha) is fused to a **mutant** form of Pseudomonas exotoxin, is selectively cytotoxic to cells bearing epidermal growth factor (EGF) receptors. Heparin binding EGF-like growth factor is a potent mitogen for smooth muscle cells capable of binding to both the EGF receptor and to immobilized heparin (Higashiyama, S., Abraham, J., Miller, J., Fiddes, J., and Klagsbrun, M. (1991) Science 251, 936-938). To study the effect of the heparin-binding domain in a chimeric toxin targeted to the EGF receptor, we fused the DNA sequence corresponding to the putative NH-2-terminal heparin-binding (HB) domain of HB-EGF to chimeric toxins composed of TGF-alpha and two different recombinant forms of Pseudomonas exotoxin (PE). One of these is a truncated form of PE devoid of the binding domain (TGF-alpha-PE38); another is a **mutant** form of full-length **toxin** containing inactivating **mutations** in the binding domain and an altered carboxyl terminus (TGF-alpha-PE-4EKDEL). The resulting chimeric toxins HB-TGF-alpha-PE38 and HB-TGF-alpha-PE-4EKDEL were expressed in Escherichia coli as inclusion bodies, refolded, and purified by heparin affinity chromatography. Both of the toxins were eluted from heparin at 0.8 M NaCl, in contrast to their respective TGF-alpha toxins which were eluted at 0.15 M. Binding studies on A431 cells showed that the HB-TGF-alpha toxins bound to the EGF receptor with an affinity similar to that of the TGF-alpha toxins. However, cell killing studies on a panel of malignant cell lines showed that cytotoxicity was strongly affected by the presence of the HB domain. Cell lines expressing high numbers of EGF receptors such as A431 and KB were less sensitive to toxins containing the HB domain. Cells with low number of EGF receptors had similar responses to both types of toxins (MCF-7 and LNCaP) or were more sensitive to the toxin with the added HB domain (HEP-G2). HB-TGF-alpha-PE-4KDEL was over 10-fold more cytotoxic against proliferating vascular smooth muscle cells (VSMC) than to quiescent VSMC. Moreover, HB-TGFa-PE-4EKDEL was 6-fold more potent than TGF-alpha-PE-4EKDEL to proliferating VSMC. Competition studies with EGF and/or heparin showed that heparin blocks the cytotoxicity of HB-TGF toxins and the inhibitory action of heparin is stronger in cells expressing lower number of EGF receptors. In addition, experiments with heparitinase-treated cells showed that in cells with low numbers of EGF receptors the binding of the HB domain to cell surface heparan sulfate proteoglycans appears to facilitate the internalization of the toxin. We conclude that addition of a HB domain to TGF-alpha-PE38 or TGF-alpha-PE-4EKDEL confers the ability to bind to and to be modulated by heparin-like molecules and **increases** their **cytotoxicity** to cells expressing low numbers of EGF receptor and proliferating smooth muscle cells.

L4 ANSWER 16 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:190968 BIOSIS
DOCUMENT NUMBER: PREV199293101918; BA93:101918
TITLE: PROPERTIES OF CHIMERIC TOXINS WITH TWO RECOGNITION DOMAINS INTERLEUKIN 6 AND TRANSFORMING GROWTH FACTOR ALPHA AT DIFFERENT LOCATIONS IN PSEUDOMONAS EXOTOXIN.
AUTHOR(S): KREITMAN R J [Reprint author]; SIEGALL C B; CHAUDHARY V K; FITZGERALD D J; PASTAN I

CORPORATE SOURCE: LAB MOLECULAR BIOLOGY, DCBDC, NATL CANCER INST, NATL INST HEALTH, BETHESDA, MARYLAND 20892, USA
SOURCE: Bioconjugate Chemistry, (1992) Vol. 3, No. 1, pp. 63-68. CODEN: BCCHES. ISSN: 1043-1802.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 13 Apr 1992
Last Updated on STN: 14 Apr 1992

AB Pseudomonas exotoxin (PE) is a potent cytotoxic agent that is composed of 613 amino acids arranged into three major domains. We have previously identified two positions where ligands can successfully be placed in PE to direct it to cells with specific surface receptors. One site is at the amino terminus and the other is close to but not at the C-terminus. To examine the possibility of constructing oncotoxins with two different recognition elements that will bind to two different receptors, we have placed cDNAs encoding either transforming growth factor α (TGFα) or interleukin 6 (IL6) at the 5'end of a PE gene and also inserted a cDNA encoding TGFα near the 3' end of the PE gene. The plasmids encoding these chimeric toxins were expressed in Escherichia coli and the chimeric proteins purified to near homogeneity. In all the new toxins, the TGFα near the C-terminus was inserted after amino acid 607 of PE and followed by amino acids 604-613 so that the correct PE C-terminus (REDLK) was preserved. For each chimera, the **toxin** portion was either PE4E, in which the cell binding domain (domain Ia) is **mutated**, PE40, in which domain Ia is deleted, or PE38, in which domain Ia and part of domain Ib are deleted. These derivatives of PE do not bind to the PE receptor and allow 607, 355, or 339 amino acids, respectively, between the two ligands. Chimeric toxins containing two TGFα ligands were all cytotoxic to human cancer cells expressing EGF receptors, while those containing one IL6 and one TGFα ligand were cytotoxic toward cells expressing either IL6 or EGF receptors, or both. The effect of distance separating the two ligands was evaluated using cytotoxicity assays and [125I]EGF displacement assays. The animal toxicity of two of the bifunctional chimeric toxins was investigated in mice. Our results establish that two ligands can be placed in different locations within PE simultaneously and that adding IL6 to the amino terminus of PE which already contains TGFα near the carboxyl terminus decreases animal **toxicity** in vivo and yet **increases cytotoxicity** against some cell lines in vitro.

L4 ANSWER 17 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1986:339088 BIOSIS
DOCUMENT NUMBER: PREV198682053292; BA82:53292
TITLE: MUTAGENICITY OF QUERCETIN IN CHINESE HAMSTER LUNG CELLS IN CULTURE.
AUTHOR(S): NAKAYASU M [Reprint author]; SAKAMOTO H; TERADA M; NAGAO M; SUGIMURA T
CORPORATE SOURCE: NATIONAL CANCER CENTER RESEARCH INSTITUTE, 1-1, TSUKIJI 5 CHOME, CHUO-KU, TOKYO 104, JAPAN
SOURCE: Mutation Research, (1986) Vol. 174, No. 1, pp. 79-83. CODEN: MUREAV. ISSN: 0027-5107.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 22 Aug 1986
Last Updated on STN: 22 Aug 1986

AB Quercetin, a flavonol that is widely distributed in edible plants, was assayed for cytotoxic and mutagenic activities on Chinese hamster lung cells in culture, using diphtheria toxin resistance as a selective marker. Result showed that it was **cytotoxic**, and induced a

dose-dependent **increase** in the number of diphtheria-toxin-resistant mutants in the absence of a metabolic activation system.

L4 ANSWER 18 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1986:93408 BIOSIS
DOCUMENT NUMBER: PREV198681003824; BA81:3824
TITLE: GENETIC EFFECTS OF PR TOXIN ON PROKARYOTIC AND EUKARYOTIC MICROORGANISMS.
AUTHOR(S): RAVENNA L [Reprint author]; MORACE G; POLONELLI L
CORPORATE SOURCE: ISTITUTO DI MICROBIOLOGIA DELLA FACOLTA DI MEDICINA E CHIRURGIA-LGO F VITO, 1-00168 ROMA, ITALY
SOURCE: Igiene Moderna, (1985) Vol. 83, No. 4, pp. 646-655. CODEN: IGMPAX. ISSN: 0019-1655.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ITALIAN
ENTRY DATE: Entered STN: 25 Apr 1986
Last Updated on STN: 25 Apr 1986

AB The mutagenic and convertogenic activity of PR toxin, a mycotoxin produced from cultures of Penicillium roqueforti, was tested on prokaryotic and eukaryotic microorganisms. The following short-term tests were peformed: Salmonella typhimurium microsome test (preincubation method), mitotic gene conversion test on Saccharomyces cerevisiae D4 with growing and stationary cells and point mutation test on Schizosaccharomyces pombe P1. A weak genotoxicity was observed on Salmonella typhimurium TA 98 in the test without metabolic activation at doses toxic to the microorganism. In the forward **mutation** system of S. pombe, PR **toxin** failed to show any mutagenic activity. In the mitotic gene conversion test on S. cerevisiae D4, a slight **increase** in trp+ convertant frequency was observed at highly **toxic** doses but the results need further studies to assert the convertogenic activity of PR toxin.

L4 ANSWER 19 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:210063 BIOSIS
DOCUMENT NUMBER: PREV198477043047; BA77:43047
TITLE: CYTO TOXICITY AND ABSENCE OF MUTAGENIC ACTIVITY OF VOMI **TOXIN** 4 DEOXY NIVALENOL IN A HEPATOCYTE MEDIATED **MUTATION** ASSAY WITH V-79 CHINESE HAMSTER LUNG CELLS.
AUTHOR(S): ROGERS C G [Reprint author]; HEROUX-METCALF C
CORPORATE SOURCE: TOXICOL RES DIV, HEALTH PROTECTION BRANCH, HEALTH AND WELFARE CAN, OTTAWA, K1A 0L2, CAN
SOURCE: Cancer Letters, (1983) Vol. 20, No. 1, pp. 29-36. CODEN: CALEDQ. ISSN: 0304-3835.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Cytotoxicity and mutagenicity of vomitoxin (4-deoxynivalenol), a tricothecene mycotoxin produced on cereal grains by fungi of the genus Fusarium, were determined in vitro with Chinese hamster V79 cells. Cytotoxicity was shown by a reduction in colony size at 1 μg/ml (ppm); by reduction in the number and size of colonies at 2-3 μg/ml or higher; and by lethality to 80-90% of the cells at 10 μg/ml. Up to 3 μg/ml, vomitoxin was non-mutangenic to V79 cells at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus, with or without hepatocyte-mediated activation; and did not significantly **increase** the number of 6-thioguanine-resistant mutants at marginally **cytotoxic** levels of 6 and 8 μg/ml (data not shown). Vomitoxin, like other 12,13-epoxytricothecenes, may become cytotoxic through inhibition of protein and /or DNA synthesis, and is likely to be

non-carcinogenic.

L4 ANSWER 20 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1982:237392 BIOSIS
DOCUMENT NUMBER: PREV198274009872; BA74:9872
TITLE: TESTS FOR MUTAGENICITY IN SALMONELLA AND COVALENT BINDING TO DNA AND PROTEIN IN THE RAT OF THE RIOT CONTROL AGENT O CHLOROBENZYLIDENE MALONONITRILE.
AUTHOR(S): VON DANIKEN A [Reprint author]; FRIEDERICH U; LUTZ W K; SCHLATTER C
CORPORATE SOURCE: INST TOXICOL, SWISS FEDERAL INST TECHNOL, CH-8603 SCHWERZENBACH, SWITZERLAND
SOURCE: Archives of Toxicology, (1981) Vol. 49, No. 1, pp. 15-28. CODEN: ARTODN. ISSN: 0340-5761.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB o-Chlorobenzylidene malononitrile (CS) was studied for any genotoxic activity towards Salmonella or mammalian DNA in vivo. CS was synthesized with a [14C]-label at the benzylic carbon atom. It was administered i.p. at a dose level of 13 mg/kg (1 mCi/kg) to young adult male rats. Liver and kidney DNA was isolated after 8, 25 and 75 h. The radioactivity was at (liver, 8 and 75 h) or below (all other samples) the limit of detection of 3 dpm. A possible binding of CS to DNA is at least 105 times lower than the strong hepatocarcinogen aflatoxin B1, and 4000 times lower than vinyl chloride. In contrast to this lack of DNA binding, but in agreement with the chemical reactivity of CS, a binding to nuclear proteins could be detected with specific activities ranging between 50-121 dpm/mg for liver and between 3 and 41 dpm/mg for kidney. Protein binding could well be responsible for its pronounced cytotoxic effects. CS was also tested in the Ames Salmonella/microsome assay. Strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were used with or without pre-incubation. Only with strain TA 100 and only without pre-incubation, a doubling of the number of revertants was detectable at the highest dose levels used, 1000 and 2000 µg CS/plate. With pre-incubation of TA 100 with CS, a slight increase of the number of revertants was seen at 100 and 500 µg/plate, and a subsequent fall below control values at 1000 µg. A check for the number of surviving bacteria revealed a strong bacteriotoxicity of the higher doses of CS so that the calculated mutation frequencies, the number of revertants/number of surviving bacteria, increased with doses up to 500 µg. This **toxicity** could be counteracted in part by the addition of **increasing** amounts of rat liver microsomes. Taking into account the rare and low exposure of man, CS will not create a risk for the induction of point mutations or of carcinogenic processes mediated by DNA binding.

L4 ANSWER 21 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1994:105024 CAPLUS
DOCUMENT NUMBER: 120:105024
TITLE: Mutant cytokines having increased receptor affinity
INVENTOR(S): Lakkis, Fadi; Murphy, John R.
PATENT ASSIGNEE(S): University Hospital, USA
SOURCE: PCT Int. Appl., 28 pp. CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9321308 A1 19931028 WO 1993-US3613 19930416
W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9342891 A1 19931118 AU 1993-42891 19930416
PRIORITY APPLN. INFO.: US 1992-870500 A 19920417
WO 1993-US3613 A 19930416

AB A variant of a naturally-occurring cytokine has a neutral amino acid substituted for a neg.-charged amino acid within 2 amino acids immediately upstream or downstream from a Phe-Leu or Tyr-Leu sequence in a helical domain. The variant cytokine has an increased affinity for the receptor. A hybrid mol. comprises a receptor-binding portion of the variant cytokine joined together covalently with a mol. having enzymic activity (e.g., a cytotoxin). The hybrid mol. decreases cell viability. DAB389-mIL-4, a fusion protein containing diphtheria toxin having a deletion of 97 amino acids (Thr387-His485; the generalized cell binding domain) replaced with murine IL-4, was altered by site-directed and in-frame deletion mutagenesis to alter the mIL-4 portion of DAB389-mIL-4. Deletion of the C-terminal 15 amino acids of mIL-4; substitution of Phe496 with Pro, Ala, or Tyr; or substitution of Leu497 with Ala or Glu decreased binding to the mIL-4 receptor and cytotoxicity. In contrast, the substitution of the neg.-charged residue Asp495 with Asn resulted in a 4-fold **increase** in **cytotoxic** potency and binding affinity to mIL-4 receptor bearing cells in vitro.

L4 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:493090 CAPLUS
DOCUMENT NUMBER: 113:93090
TITLE: Construction by site-directed mutagenesis of a 39-kilodalton mosquitocidal protein similar to the larva-processed toxin of Bacillus sphaericus 2362
AUTHOR(S): Broadwell, Andrew H.; Clark, Marta A.; Baumann, Linda; Baumann, Paul
CORPORATE SOURCE: Dep. Microbiol., Univ. California, Davis, CA, 95616, USA
SOURCE: Journal of Bacteriology (1990), 172(7), 4032-6
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB After ingestion of the parasporal crystals of B. sphaericus, mosquito larvae process the 42-kilodalton (kDA) toxin to a protein of 39 kDa, which has an **increased toxicity**. A similar activation is performed by trypsin and chymotrypsin. Using site-directed mutagenesis, derivs. of the 42-kDa toxin were constructed with a deletion of 10 amino acids at the N terminus and deletions of 7, 17, or 20 amino acids at the C terminus. Toxicity for mosquito larvae was retained upon deletion of 7 or 17 amino acids but was lost upon deletion of 20 amino acids. Evidence is presented indicating that the protein containing deletions of 10 amino acids at the N terminus and 17 amino acids at the C terminus (corresponding to potential chymotrypsin cleavage sites) is similar to the 39-kDa protein produced in mosquito larvae or by digestion with chymotrypsin. Digestion with trypsin appears to generate a protein lacking 16 or 19 amino acids from the N terminus and 7 amino acids from the C terminus. As is the case with the recombinant-made 42-kDa protein, toxicity of its derivs. is dependent on the presence of a 51-kDa protein which is a component of the parasporal crystal of B. sphaericus 2362.

L4 ANSWER 23 OF 23 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 1997-424316 [39] WPIDS
DOC. NO. NON-CPI: N1997-353476

DOC. NO. CPI: C1997-135796
TITLE: Modified Bacillus thuringiensis CryIII proteins - with **increased toxicity** against insect pests, particularly Coleopteran insects, e.g. corn rootworm and Colorado potato beetle.
DERWENT CLASS: C05 C06 D16 P13
INVENTOR(S): JANSENS, S; PEFEROEN, M; VAN, RIE J
PATENT ASSIGNEE(S): (PLBZ) PLANT GENETIC SYSTEMS NV
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5659123	A	19970819	(199739)*		22

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 5659123	A	US 1994-295060	19940826

PRIORITY APPLN. INFO: US 1994-295060 19940826
AN 1997-424316 [39] WPIDS
AB US 5659123 A UPAB: 19970926
Novel recombinant or synthetic DNA sequence (A) encodes a modified CryIII protein with an **increased toxicity** to a Coleopteran insect, where an amino acid occurring in at least 1 position is replaced by a different amino acid, the replaced amino acid being located, or structurally analogous to an amino acid located: (a) in domain II and having a relative solvent accessibility of at least 40%; or (b) at a maximum distance, in the primary sequence, of 3 amino acids from an amino acid having at least 40% relative solvent accessibility in domain II. Also claimed are: (1) plant or seed including incorporated in its genome, a chimeric gene comprising (A) operably linked to regulatory sequences allowing expression in cells of the plant; (2) modified CryIII protein encoded by (A); (3) improving the **toxicity** of a CryIIIA protein for corn rootworm pests, comprising replacing at least 1 of the amino acids marked with Xaa in the 645 residue amino acid sequence given in the specification by another amino acid; and (4) DNA sequence encoding at least a modified CryIIIA **toxin** with improved **toxicity** to the Colorado potato beetle, having the 644 residue amino acid sequence given in the specification, comprising the **mutation** Pro348Ala.
USE - The modified CryIII proteins can be used to combat, or improve plant resistance towards insects, particulary Coleopteran insects, e.g. corn rootworm or Colorado potato beetle.
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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	89.12	89.33

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-1.50	-1.50

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FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Feb 10, 2006 (20060210/UP).

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(FILE 'HOME' ENTERED AT 21:14:03 ON 16 FEB 2006)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, WPIDS' ENTERED AT 21:14:19 ON 16 FEB 2006

L1 133 ((MUTAT? OR MUTANT) (S) TOXIN) AND ((CYTOTOX? OR TOXIC OR TOXIC
L2 68 DUP REM L1 (65 DUPLICATES REMOVED)
L3 45 PY>1998 AND L2
L4 23 L2 NOT L3

FILE 'STNGUIDE' ENTERED AT 21:23:05 ON 16 FEB 2006

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L5 0 .

=> logoff y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	2.34	91.67

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	0.00	-1.50

STN INTERNATIONAL LOGOFF AT 21:46:15 ON 16 FEB 2006